BIOSENSORS HAVING IMPROVED SAMPLE APPLICATION AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to biosensors having improved sample application and measuring properties and their uses for detection, preferably, quantitative measurement, of analyte or enzyme in a liquid sample. In particular, the invention provides for a biosensor having a configuration and reagents facilitating the speed and uniformity of sample application, especially small volume sample application. Methods for assaying analytes or enzymes using the biosensors are further provided.

BACKGROUND OF THE INVENTION

A biosensor is an analytical device that comprises at least two components: an immobilized biological component responsible for the selective recognition of the test species and a suitable transducer device responsible for relaying the biological signals for further analysis. Among others, electrochemical biosensors that employ biological recognition systems and electrochemical transudation offer a possibility of quick and real-time analysis, which is particularly suited for the rapid measurement of point-of-care industry. The evolution of these devices comes from the multi-discipline of electronics, material science, electrochemistry, biochemistry, and immunochemistry. The technology of electroanalysis is an interplay between electricity and chemistry that concerns current, potential, and charge from a chemical reaction. There are two principal types of electroanalytical measurements, potentiometric and amperometric. Potentiometric technique is a static technique with no current flow; the established potential across the ion-select membrane is measured. With different types of membrane materials, the recognition of different ions can be reached. Thus, the potentiometric probes have been widely used for directly monitoring ionic species such as calcium, potassium, and fluoride ions. In amperometric technique, an electrode potential is used to drive an electron-transfer reaction. The responsive current is measured and related to the presence and/or concentration of the target analyte. In the past, potentiometric devices have been more widely applied in clinical chemistry laboratories. But with increasing amount of research on amperometric systems in diagnostics, the balance has shifted. The amperometric biosensors make possible a practical, fast, and routine measurement of test analytes. One trend of current biosensors focuses on the methodology of minimum demand of operator skills.

To date, most commercially used biosensors are amperometric ones that harness redox enzymes as recognizing biocomponents and electrodes as electrochemical transducers. The mass production of inexpensive and disposable devices has been achieved recently with the help of screen-printing technology. The success in the development of these devices has led to amperometric assays for several biomolecules including glucose, cholesterol, and various drugs. This type of amperometric biosensor is typically composed of an base member, two or three electrodes, an insulating layer, and a region for enzymatic reaction. Two-electrode biosensor consists of a working electrode, a counter electrode and a destined region where reagent for enzymatic reaction is placed. The reaction progresses when the sample liquid containing an analyte is applied onto the reaction area. Two physical effects, mesh spread and capillary action, are commonly used to guide a uniform distribution of the loaded

sample on the reaction area. After the reaction is complete, the test analyte is oxidized and the electrons yielded from the reaction are trapped in a reduced co-product. A controlled-potential is then applied between the electrodes to trigger a second round of oxidoreduction. This electrical potential must be sufficient enough to drive a diffusion-limited electrooxidation at the surface of the working electrode, yet insufficient to activate irrelevant chemical reactions. After a time delay, the current produced by the electrochemical oxidoreduction is observed and measured and the current is correlated to the presence and/or amount of the analyte in the sample.

In the case of oxidation, oxygen is consumed in the oxidative reaction as a co-reactant and hydrogen peroxide is yielded as a co-product. The yield of hydrogen peroxide is proportional to the concentration of analyte. Hydrogen peroxide can be detected by oxidizing it at anodic potential (e.g., >0.6 V, Ag/AgCl) to generate an electrical signal (current). However, the potential required for oxidizing hydrogen peroxide can cause oxidation of other oxidizable chemicals such as ascorbate, bilirubin, uric acid, and the commonly used drug, e.g., acetaminophen, thus leading to an interference of electrical current to be detected. This interference can be avoided by replacing oxygen with an artificial mediator capable of transferring electrons from oxidoreductases. Several mediators have been used to enhance electron transfer between a variety of enzymes and electrodes, which include ferrocene and its derivatives, osmium complex, tetrathiofulvalene, phenazine ethosulfate, benzoquinone, and hexacyanoferrate.

Conventional methods of determining analytes in blood involves sample pretreatment. However, as pretreatment involves extra time and labor, these assays may benefit through the availability of a direct measurement of whole blood samples. More importantly, direct measurement of whole blood samples makes it possible for a real time monitoring for home users. For accurate measurement of a whole blood sample using an amperometric biosensor, a quick and homogenous reaction on the electrodes is essential for successful analyte determination. Reagents dried on a reaction area, including an oxidoreductase and a mediator, have to dissolve instantly when a small volume of sample blood is applied to the biosensor. These dissolved reagents must mix with the sample thoroughly for the completion of the enzymatic reaction and consistency of the subsequent electronic reaction.

The other common problems for assaying biological samples such as the whole blood are sample viscosity and the relatively large sample volume for the analysis. The whole blood sample, with its viscosity, might not be able to be distributed over sufficient reaction area. Moreover, viscosity and surface tension of samples may present a lag-time in sample introduction to the reaction area in biosensors, thus inhibiting real-time analysis of particular analytes. For some poorly bleeding people, it might be a problem to get enough blood from a prick on fingerstick. Three types of insufficient application of blood (or other viscous samples) have been observed: first, the sample covers only the front end of the test strip; secondly, the sample covers only the right half of the strip; and thirdly, the sample covers only the left half of the strip. The insufficient or non-homogenous application of sample fluid presents a lower amount of analyte, which causes an artificial and misleading result.

Moreover, with particular regard to glucose assays, current devices may only provide accurate results within a particular glucose range. This range may lie outside and